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Vasinfectins A and B: New Phytotoxins from *Neocosmospora vasinfecta*

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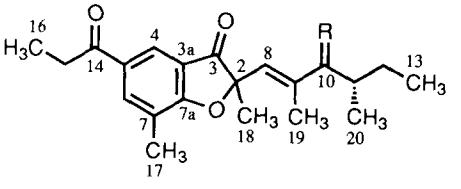
Abstract: Two new phytotoxins, vasinfectins A and B, were isolated from the phytopathogenic fungus *Neocosmospora vasinfecta* NHL2298. Their structures, diastereomers of each other, were determined to be (*E*)-2-(3-hydroxy-2,4-dimethyl-1-hexenyl)-2,7-dimethyl-5-propanoyl-2*H*-benzo[*b*]furan-3-one by 2D NMR. The absolute stereochemistry of C-10 and C-11 in these vasinfectins was established by chemical reactions. © 1997 Elsevier Science Ltd.

Neocosmospora vasinfecta E.F.Smith is a pathogen which causes root- and fruit-rot and seedling damping-off in the Malvaceae, Leguminosae, Piperaceae and Cucurbitaceae.[1] In our search for phytotoxic compounds among the metabolites of phytopathogens, neovasinin, isolated from a strain (NHL2298) of *N. vasinfecta*, was found to be phytotoxic to soybean, a host plant of this fungus.[2] During our continued chemical research, neovasipyrones and neovasifuranones related to neovasinin have been isolated from the same fungus and characterized.[3] We also found that this fungus produces two new phytotoxic metabolites with a benzofuranone ring, vasinfectins A and B, which cause chlorosis at 0.3 μg/leaf on young soybean (*Glycine max* L.) leaves in the leaf spot assay. We here report the isolation and determination of their structures.

Vasinfectins A (**1a**) and B (**1b**) were isolated by several chromatographies as oils in respective yields of 0.48 and 0.50 mg/l from a culture filtrate of *N. vasinfecta* NHL2298.[4] The ^{13}C NMR and HREIMS spectra[5] of **1a** gave the molecular formula $C_{21}H_{28}O_4$ (8 unsaturations). The ^{13}C NMR spectrum of **1a** has 21 resonances: six owing to methyl groups, two to methylenes, five to methines and eight to quaternary carbons from its DEPT data. Acetylation of **1a** afforded a monoacetyl derivative [**2**: δ_H 4.99 (br.d, 5.9Hz, 10-H), 2.06 (s, Ac); EIMS *m/z*: 386 (M^+)], confirming that one of the protons in the molecule is bound to oxygen.



1a R = α-OH, β-H **2** R = α-OAc, β-H
1b R = α-H, β-OH **3** R = O

The structural fragments of **1a** (Fig.1) were deduced from ^1H-^1H COSY, ^{13}C-^1H COSY, COLOC experiments and a consideration of chemical shift. The *E* geometry of the olefinic bond (C-8 and C-9) was confirmed by DIFNOE data; irradiation of the olefinic proton 8-H (δ_H 5.56) caused NOE enhancement of the methine proton signal 10-H (δ_H 3.77). The connections of these fragments

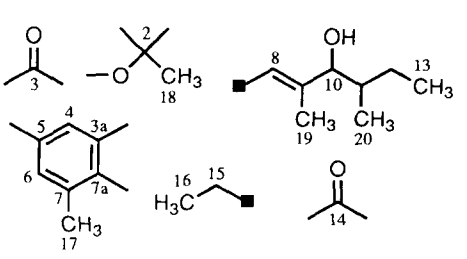
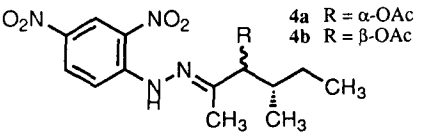


Fig. 1. Structural fragments of **1a**

were deduced from the COLOC correlations (C-2, C-3, C-8/18-H_3; C-14/4-H, 6-H, 15-H_2; C-3/4-H). The unsaturation of **1a** and chemical shift of the aromatic carbon C-7a (δ_C 172.6) indicated a bond between C-7a and the oxygen atom on C-2. Thus, the planar structure for **1a** was established.

Vasinfectin B (**1b**) had the same molecular formula as **1a**, and its spectral features[6] are very similar to those of **1a**. The geometry of its double bond, *E*, was confirmed by DIFNOE data. These findings suggested that **1b** is a diastereomer of **1a**. Oxidation of **1a** with DMSO-Ac_2O[7] gave a 10-oxo derivative (**3**),[8] which was identical with the derivative from **1b**. Thus, **1b** is a diastereomer of **1a** differing only in its stereochemistry at C-10. The stereochemistries of C-10 and C-11 in **1** were determined by chemical reactions. Acetylation of **1a** with Ac_2O-Py gave **2**. The reaction of **2** with $RuCl_3$-$NaIO_4$,[9] followed by treatment of the product with DNPH, afforded **4a** (Fig.2).[8] The same treatments of **1b** gave **4b** (Fig.2).[8] Similar reactions of neovasipyrones A and B, whose stereochemistries were established,[3] respectively gave the same products, **4a** and **4b**. The configurations of C-10 and C-11 in **1a** therefore are (*R*, *S*) and in **1b** are (*S*, *S*). The stereochemistry of C-2 has yet to be resolved.



4a R = α-OAc
4b R = β-OAc

Fig. 2. Structures of **4a** and **4b**

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4. For the extraction of the metabolites see ref. 3. Silica gel C. C. (10% acetone in hexane) of the EtOAc-soluble neutral fr. and silica gel TLC (5% acetone in $CHCl_3$) of the 10% fr. gave a mixture of vasinfectins. Vasinfectins A and B were separated by reversed phase HPLC (Cosmosil $5C_{18}$-AR, 75% MeOH).
5. **1a**: colorless oil; HREIMS: found *m/z* 344.1983, calcd. for $C_{21}H_{28}O_4$ *m/z* 344.1988; $[\alpha]_D^{20}$ –53° (*c* 0.5, EtOH); IR ν_{max} (film): 3434, 1725, 1686, 1607 cm^{-1}; UV λ_{max} (EtOH): 202 nm (ε 11,000), 243 (35,500), 274 (9300), 329 (3500); ^1H NMR ($CDCl_3$): δ 8.11 (dq, 1.8, 0.9Hz, 6-H), 8.07 (d, 1.8Hz, 4-H), 5.56 (dq, 1.1, 1.1Hz, 8-H), 3.77 (br.d, 6.1Hz, 10-H), 2.94 (q, 7.3Hz, 15-H_2), 2.33 (br.s, 17-H_3), 1.66 (d, 1.1Hz, 19-H_3), 1.59 (s, 18-H_3), 1.50 (m, 11-H), 1.34, 1.10 (m, 12-H_2), 1.19 (t, 7.3Hz, 16-H_3), 0.88 (t, 7.3Hz, 13-H_3), 0.82 (d, 6.8Hz, 20-H_3); ^{13}C NMR ($CDCl_3$): δ 202.4 (C-3), 198.9 (C-14), 172.6 (C-7a), 144.0 (C-9), 137.9 (C-6), 131.3 (C-5), 124.1 (C-7), 123.1 (C-4), 122.6 (C-8), 118.8 (C-3a), 90.8 (C-2), 80.7 (C-10), 37.4 (C-11), 31.5 (C-15), 26.3 (C-12), 24.0 (C-18), 14.3 (C-17), 13.7 (C-19), 13.6 (C-20), 11.6 (C-13), 8.4 (C-16).
6. **1b**: colorless oil; HREIMS: found *m/z* 344.1992, calcd. for $C_{21}H_{28}O_4$ *m/z* 344.1988; $[\alpha]_D^{20}$ –41° (*c* 0.5, EtOH); IR ν_{max} (film): 3499, 1726, 1686, 1607 cm^{-1}; UV λ_{max} (EtOH): 202 nm (ε 11,300), 243 (36,000), 274 (9400), 329 (3500); ^1H NMR ($CDCl_3$): δ 8.14 (dq, 1.8, 0.9Hz, 6-H), 8.10 (d, 1.8Hz, 4-H), 5.55 (dq, 1.2, 1.2Hz, 8-H), 3.68 (br.d, 7.7Hz, 10-H), 2.97 (q, 7.3Hz, 15-H_2), 2.36 (br.s, 17-H_3), 1.68 (d, 1.2Hz, 19-H_3), 1.62 (s, 18-H_3), 1.51 (m, 11-H), 1.66, 1.11 (m, 12-H_2), 1.21 (t, 7.3Hz, 16-H_3), 0.88 (t, 7.3Hz, 13-H_3), 0.72 (d, 6.8Hz, 20-H_3); ^{13}C NMR ($CDCl_3$): δ 202.3 (C-3), 199.0 (C-14), 172.5 (C-7a), 144.1 (C-9), 138.0 (C-6), 131.2 (C-5), 124.2 (C-7), 123.4 (C-8), 123.1 (C-4), 118.8 (C-3a), 90.7 (C-2), 82.1 (C-10), 37.4 (C-11), 31.5 (C-15), 24.3 (C-12), 24.0 (C-18), 15.6 (C-20), 14.3 (C-17), 12.9 (C-19), 11.1 (C-13), 8.4 (C-16).
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8. For **3**: IR ν_{max} (film): 1728, 1684, 1607 cm^{-1}; CD λ_{ext} (EtOH): 342 nm (Δε +6.6), 311 (–3.5), 245 (-21.3); ^1H NMR ($CDCl_3$): δ 8.17 (1H, dq, 1.8, 0.9Hz), 8.12 (1H, d, 1.8Hz), 6.53 (1H, q, 1.3Hz), 3.09 (1H, ddq, 6.8, 6.8, 6.8Hz), 2.97 (2H, q, 7.3Hz), 2.39 (3H, br.s), 2.01 (3H, br.d, 1.3Hz), 1.69 (3H, s), 1.63 (1H, m), 1.34 (1H, m), 1.22 (3H, t, 7.3Hz), 1.07 (3H, d, 6.8Hz), 0.80 (3H, t, 7.4Hz); EIMS *m/z*: 342 (M^+). For **4a**: $[\alpha]_D^{20}$ +26.2° (*c* 0.3, EtOH); IR ν_{max} (film): 1742, 1618, 1339 cm^{-1}; ^1H NMR ($CDCl_3$): δ 11.05 (1H, br.s), 9.13 (1H, d, 2.6Hz), 8.33 (1H, ddd, 9.5, 2.6, 0.5Hz), 7.95 (1H, d, 9.5Hz), 5.25 (1H, d, 7.0Hz), 2.14 (3H, s), 2.04 (3H, s), 1.91 (1H, m), 1.43 (1H, m), 1.22 (1H, m), 0.99 (3H, d, 6.7Hz), 0.95 (3H, t, 7.4Hz); EIMS *m/z*: 352 (M^+). For **4b**: $[\alpha]_D^{20}$ +16.0° (*c* 0.3, EtOH); IR ν_{max} (film): 1742, 1618, 1339 cm^{-1}; ^1H NMR ($CDCl_3$): δ 11.05 (1H, br.s), 9.13 (1H, d, 2.6Hz), 8.33 (1H, ddd, 9.5, 2.6, 0.5Hz), 7.97 (1H, d, 9.5Hz), 5.20 (1H, d, 8.2Hz), 2.13 (3H, s), 2.04 (3H, s), 1.91 (1H, m), 1.60 (1H, m), 1.24 (1H, m), 0.95 (3H, t, 7.4Hz), 0.90 (3H, d, 6.9Hz); EIMS *m/z*: 352 (M^+).
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